m.p. appeared to be sensitive to trace impurities. The m.p.'s were sharp (less than 0.5° range) and were accompanied by decomposition, but values for the same sample or different samples ranged between 105 and 130°.

Anal. Calcd. for $C_{34}H_{54}O_3S$: C, 75.23; H, 10.03. Found: C, 75.18; H, 10.19.

The compound decomposed to *p*-toluenesulfonic acid and a one to one mixture of Δ^2- and Δ^3-cholestene when dissolved in petroleum ether and passed over a column of alumina. The same type of reaction was obtained with the tosylate of 12(α)-hydroxypregnane-3,20-dione by Ruff and Reichstein.[2b]

Reaction of β-Cholestanyl Tosylate with Methanol.—A suspension of 500 mg. (0.92 millimole) of β-cholestanyl tosylate in 38 ml. of methanol (Baker and Adamson, A.C.S. Reagent Grade) was heated under reflux. Solution was complete in approximately 20 hours. After 73 hours the solvent was removed under reduced pressure and the residue taken up in water and ether. The ether layer was washed with water, dilute sodium carbonate solution, water and saturated sodium chloride solution, and then filtered through anhydrous sodium sulfate. After removal of the ether 352 mg. of colorless oil was obtained, which crystallized on standing. (Crystallization of the product of another experiment from 3 to 1 methanol–acetone gave needles, m.p. 45–70°.) The solid was taken up in 15 ml. of petroleum ether (b.p. 30–60°) and chromatographed on 16 g. of alumina in a column 20 × 70 mm. Fractions of 10 ml. were collected.

Fractions 1 (8 mg.) and 2 (43 mg.) crystallized spontaneously. Fraction 3 (40 mg., colorless oil, $[\alpha]_D$ +31°) was taken up in 10 ml. of petroleum ether and rechromatographed on 3 g. of alumina in a column 12 × 37 mm. Fractions of 5 ml. were collected. Fraction 1-A (8 mg. $[\alpha]_D$ +63°) was combined with fractions 1 and 2 above to give 57 mg. (17%) of Δ^2- and Δ^3-cholestene, $[\alpha]_D$ +65°. One half was crystallized from dilute ethanol to give 22 mg. of needles, m.p. 70.5–72°, $[\alpha]_D$ +64°; reported[8,13] for this olefin mixture from β-cholestanyl benzoate pyrolysis, 67–68°, $[\alpha]_D$ +62°. The second half was taken up in 5 ml. of carbon tetrachloride and a 5% solution of bromine in carbon tetrachloride was added until the straw color persisted. After the solution had stood overnight the solvent was removed under reduced pressure, and the residue flushed once with carbon tetrachloride to give 50.2 mg., $[\alpha]_D$ +46° (calculated[13] for 1-1 mixture of 2,3- and 3,4-dibromide, +41°).

(13) D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 2459 (1949).

Fraction 2-A (15 mg., $[\alpha]_D$ +45°) was a colorless oil and was not investigated further. Fractions 3-A to 5-A (22 mg.) were combined with fractions 4–11 (246 mg.) to yield 272 mg. (73% calculated as α-cholestanyl methyl ether) of dendritic crystals, $[\alpha]_D$ +20°. One recrystallization from acetone gave 204 mg., m.p. 62.5–63.5°; and 26 mg., m.p. 58–61.5°. The analytical sample (one additional recrystallization) had m.p. 62.8–63.8°, $[\alpha]_D$ +18°.

Anal. Calcd. for $C_{28}H_{50}O$: C, 83.51; H, 12.52; CH_3O–, 7.71. Found: C, 83.61; H, 12.63; CH_3O–, 7.22.

A one to one mixture with β-cholestanyl methyl ether softened from 50–59°, and melted at 59–75°.

Reaction of α-Cholestanyl Tosylate with Methanol.—A mixture of 200 mg. (0.37 millimoles) of α-cholestanyl tosylate and 15 ml. of methanol was heated under reflux for 6 hours. (Solution was complete after 5 hours.) The reaction product was worked up as above to yield 141 mg. of colorless oil, $[\alpha]_D$ +44°. The oil was dissolved in 10 ml. of petroleum ether (b.p. 30–60°) and the solution chromatographed on 7 g. of alumina in a column 13 × 82 mm. The column was developed with petroleum ether and 5-ml. fractions were collected. Fractions 2–6 were combined to give, after removal of the solvent, 91.4 mg. of oil which crystallized spontaneously in rosettes of needles (yield 69%, calculated as Δ^2- and Δ^3-cholestene), $[\alpha]_D$ +59°. The entire sample was dissolved in carbon tetrachloride, a slight excess of a 5% solution of bromine in carbon tetrachloride was added, and the resulting solution allowed to stand overnight. The solvent was then removed from the light straw-colored solution, and the residue flushed twice under reduced pressure with carbon tetrachloride. The resulting light-yellow oil weighed 135 mg. (69% yield calculated for Δ^2- and Δ^3-cholestene dibromides) and had rotation $[\alpha]_D$ +45° (calculated[13] for one to one mixture, +41°).

Fractions 7–10 yielded only traces of colorless oils.

Fractions 11–22 were combined and the solvent removed to yield 34 mg. (23% yield calculated as β-methoxycholestane) of colorless oil which crystallized spontaneously and had rotation $[\alpha]_D$ +24°. Recrystallization from acetone gave 23.5 mg. of white blades, m.p. 81–82° (substage heater, not corrected), $[\alpha]_D$ +20°; and 7 mg., m.p. 68–73° (substage heater, not corrected); reported for β-methoxycholestane, m.p., 82–83°[14], $[\alpha]_D$ +20°.[15]

(14) J. L. Dunn, I. M. Heilbron, R. F. Phipers, K. M. Samant and F. S. Spring, *ibid.*, 1576 (1934).

(15) T. Wagner-Jauregg and L. Werner, *Z. physiol. Chem.*, **213**, 123 (1932).

PROVIDENCE 12, R. I.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

A New Route to 11-Ketosteroids by Fission of a $\Delta^{9(11)}$-Ethylene Oxide. II

BY HANS HEYMANN AND LOUIS F. FIESER

RECEIVED JULY 29, 1952

3α-Hydroxy-20-keto-$\Delta^{9(11)}$-pregnene has been prepared. This ketone and methyl 3α-hydroxy-$\Delta^{9(11)}$-etiocholanate have been converted into the corresponding 11-keto derivatives. The synthesis made use of the 9,11-oxides and the 3β-hydroxy-3α-9α-oxido-11-keto derivatives.

The conversion of $\Delta^{9(11)}$-lithocholenic acid to 3-hydroxy-11-ketocholanic acid in a novel fashion *via* the ethylene oxide has been described in our first paper.[1] At present we wish to report application of the procedure to 3α-acetoxy-20-keto-$\Delta^{9(11)}$-pregnene (V) and to 3α-acetoxy-$\Delta^{9(11)}$-etiocholenic acid (I). Introduction of the C_{11}-oxygen function into the last-named compound (I) served to correlate a synthetic steroid[2] with an intermediate to cortisone, and this conversion has been the subject of a preliminary communication.[3]

The requisite olefins were prepared from a sample of 3α-hydroxy-11-ketoetiocholanic acid.[4] Sodium borohydride reduction[1] followed by acetylation furnished the 3α-acetoxy-11β-hydroxy acid, which was dehydrated with boron fluoride etherate to give 3α-acetoxy-$\Delta^{9(11)}$-etiocholenic acid (I). Methyl 3α-acetoxy-11β-hydroxyetiocholanate[5] by analogous

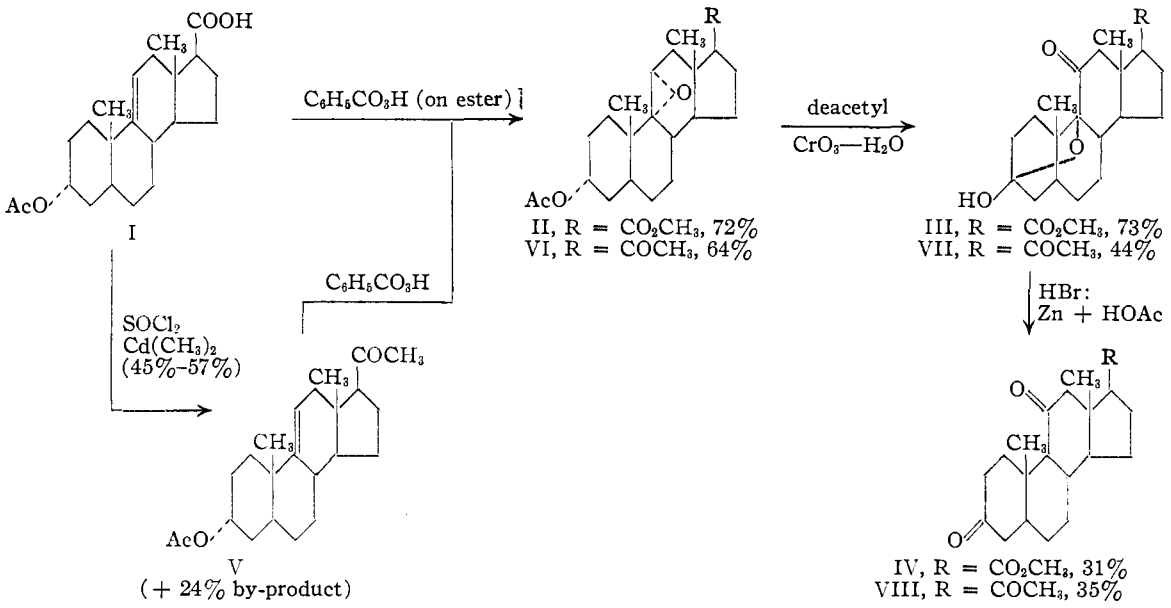
(1) Hans Heymann and L. F. Fieser, THIS JOURNAL, **73**, 5252 (1951).

(2) R. B. Woodward, F. Sondheimer and D. Taub, *ibid.*, **73**, 4057 (1951).

(3) Hans Heymann and L. F. Fieser, *ibid.*, **73**, 4054 (1951).

(4) The material was kindly supplied by Dr. Max Tishler, Merck and and Company, Inc., Rahway, New Jersey.

(5) T. Reichstein and A. Lardon, *Helv. Chim. Acta*, **26**, 705 (1943).



treatment gave the corresponding $\Delta^{9(11)}$-olefinic ester,[5] and this was converted to the 9(11)-oxido ester in 72% yield by perbenzoic acid. The ensuing operations were carried out as described before,[1] and as outlined in the diagram, yielding methyl 3,11-diketoetiocholanate (IV) as the desired final product.

For the preparation of the acetoxypregnenone V the acid chloride of I was treated with dimethylcadmium.[6] The principal by-product was the tertiary alcohol, 3α-acetoxy-20-methyl-$\Delta^{9(11)}$-pregnene-20-ol, formed by reaction of the ketonic carbonyl group with the metallo-organic reagent; a slightly better yield of V was obtained in an experiment in which the ether had not been replaced by benzene.[7] The ketone V was obtained in two polymorphic forms, m.p. 84.2–85.1° and 112.6–113.6°, and the higher melting modification had the deceptive appearance of a chromatographically different compound. However, the infrared spectra of the two compounds were nearly indistinguishable, the lower-melting form could be transformed into the higher-melting one, a mixed melting point determination showed no depression, and on hydrolysis both forms gave the same 3α-hydroxy-20-keto-$\Delta^{9(11)}$-pregnene. A small amount of a by-product was observed, resulting from the hydrolysis of the higher-melting form of V; conceivably partial inversion at C_{17} had occurred.

The remainder of the synthesis proceeded with fair yields as outlined in the diagram, and afforded the desired 3,11,20-triketopregnane (VIII).

Experimental[8]

3α-Acetoxy-11β-hydroxyetiocholanic Acid.—A solution of 10 g. of 3α-hydroxy-11-ketoetiocholanic acid[4,5] and 5 g. of sodium borohydride in 250 cc. of 0.3 *N* sodium hydroxide was boiled for one hour. The acid was precipitated by dilute acetic acid, dried, and acetylated for 17 hours with 30 cc. of pyridine and 15 cc. of acetic anhydride. Recrystallization of the product from methanol gave 6.65 g. of needles, m.p. 209.6–211.8°, and a second crop of 1.77 g., m.p. 209.5–211.0° (74.5%). The analytical sample melts 210.5–212.3°, $[\alpha]^{22}D$ + 88.3° (1.91% Chf).

Anal. Calcd. for $C_{22}H_{34}O_5$ (378.49): C, 69.81; H, 9.05. Found: C, 69.40; H, 8.89.

3α-Acetoxy-$\Delta^{9(11)}$-etiocholenic Acid (I).—To the solution of 288 mg. of 3α-acetoxy-11β-hydroxyetiocholanic acid in 2 cc. of acetic acid was added 1 cc. of boron fluoride etherate, and the mixture was allowed to stand for 4 hours at room temperature. Dilution precipitated 264 mg. (96.5%) of the product, m.p. 212–215°. The analytical sample melts at 213.2–216.1°, $[\alpha]^{22}D$ +77.2° (1.53% Chf).

Anal. Calcd. for $C_{22}H_{32}O_4$ (360.48): C, 73.30; H, 8.95. Found: C, 73.29; H, 8.87.

Methyl 3α-Acetoxy-9α,11α-oxidoetiocholanate (II).—Methyl 3α-acetoxy-11β-hydroxyetiocholanate,[5] obtainable by sodium borohydride reduction and esterification of the corresponding 3α-hydroxy-11-keto acid[4,5] was dehydrated by treatment with 10% boron fluoride etherate in acetic acid at room temperature for eight hours or on the steam-bath for 15 minutes. Crude methyl 3α-acetoxy-$\Delta^{9(11)}$-etiocholenate, m.p. 131–133.5°, resulted in 94.5% yield; recrystallized material showed the characteristic double m.p. 126–128° and 133–134°.[5] A solution of 2.25 g. of the olefin in benzene was treated with perbenzoic acid at room temperature for 18 hours. Chromatography of the product revealed the presence of unchanged starting material which was recycled. A total yield of 1.61 g. (72%) of oxido ester, m.p. 165–167°, was obtained. The pure compound forms needles from methanol, m.p. 166.9–167.4°, $[\alpha]^{23}D$ +66.4 ± 0.4° (1.4343% Chf).

Anal. Calcd. for $C_{23}H_{34}O_5$ (390.50): C, 70.74; H, 8.77. Found: C, 70.47; H, 8.74.

Methyl 3α-Hydroxy-9α,11α-oxidoetiocholanate.—A solution of 0.7 g. of the acetoxyoxido ester just described in 20 cc. of methanol 0.4 *N* in sodium methoxide was boiled for 1 hour and 25 minutes, neutralized with acetic acid and diluted with water. The crude product weighed 0.6 g. (96%), m.p. 145–148°. Recrystallization from ligroin (70–90°) containing about 20% of benzene gave silky needles, m.p. 149.8–151.0°, $[\alpha]^{25}D$ +48.9 ± 2° (1.92% Chf).

Anal. Calcd. for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26. Found: C, 72.20; H, 9.50.

Methyl 3β-Hydroxy-3α,9α-oxido-11-ketoetiocholanate (III).—A mixture of 80 mg. of the hydroxyoxido ester just described, 2 cc. of acetic acid, 0.1 g. of chromic anhydride and 1 cc. of water was allowed to stand at room temperature for 18 hours. Dilution with water furnished 46 mg. (55%) of crude III, m.p. 168–175°. Recrystallization from dilute methanol gave leaflets or chunky prisms of II, both forms

(6) J. Cason, *Chem. Revs.*, **40**, 15 (1947).

(7) *Cf.* W. Cole and P. Julian, THIS JOURNAL, **67**, 1369 (1945).

(8) Melting points are corrected. Microanalyses were carried out by Mrs. S. Golden and in the microchemical laboratory of Mr. S. Nagy at the Massachusetts Institute of Technology. All optical measurements were carried out with chloroform (Chf) or acetone (An) as the solvent. We thank Mr. K. Nakanishi for determining some of the optical data.

melting 174.6–176.0°, $[\alpha]^{25}D$ +134.6 ± 2° (2.28% Chf); λ_{max}^{chf} 2.92, 5.83, 5.86 μ.

The hemiketal III was also obtained from the 3-keto oxide described below. A solution of 613 mg. of the keto oxide in 5 cc. of acetic acid was treated with 1.25 cc. of water and 0.5 g. of chromic anhydride at room temperature for 17 hours. Dilution with water precipitated 451 mg. (70.5%) of III, m.p. 171–174°.

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.58: H, 8.34. Found: C, 69.30; H, 8.44.

The acetate was prepared by treatment of a sample of crude oxidation product with boron fluoride–acetic anhydride overnight. Chromatography and recrystallization from dilute methanol yielded long, flat needles, m.p. 123.8–125.3°, $[\alpha]^{25}D$ +135 ± 2° (2.82% Chf); no absorption at 2.8–3.0 μ.

Anal. Calcd. for $C_{23}H_{32}O_6$ (404.49): C, 68.29; H, 7.97. Found: C, 68.01; H, 8.09.

Methyl 3-Keto-9α,11α-oxidoetiocholanate.—To 48 mg. of methyl 3α-hydroxy-9α,11α-oxidoetiocholanate was added 1 cc. of acetic acid containing 20 mg. of sodium dichromate dihydrate. The solution stood at room temperature for 2.25 hours and was warmed on the steam-bath for one minute. Water precipitated 44 mg. (92%) of the ketone, m.p. 138.8–139.8°. Recrystallization from dilute acetone yielded leaflets, m.p. 128–130° and, after resolidification, 138.0–139.8°, $[\alpha]^{25}D$ +29.4 ± 2° (2.24%) Chf, λ_{max}^{chf} broad band 5.78–5.88 μ.

Anal. Calcd. for $C_{21}H_{30}O_4$ (346.45); C, 72.80; H, 8.73. Found: C, 72.62; H, 8.85.

Methyl 3,11-Diketoetiocholanate (IV).—The hemiketal III (154 mg.) was treated with dry hydrogen bromide as described before.[1] Recrystallization of the crude reaction product gave 92 mg. (51%) of methyl 3,11-diketo-12α-bromoetiocholanate,[5] m.p. 168–172°. Debromination[5] afforded 47 mg. (61%) of III, m.p. 180.6–185.6°, and, after recrystallization from methanol, 183.8–186.2°, not depressed by admixture of an authentic sample. The optical rotations for the sample prepared by the present method, and for the authentic sample are: $[\alpha]^{25}D$ +92.4 ± 2° (2.11% Chf) and $[\alpha]^{25}D$ +92.8 ± 2° (2.25% Chf). The infrared spectra of the two samples also indicated identity.

3α-Acetoxy-$\Delta^{9(11)}$-pregnene-20-one (V) and 3α-Acetoxy-20-methyl-$\Delta^{9(11)}$-pregnene-20-ol.—A mixture of 3.2 g. of 3α-acetoxy-$\Delta^{9(11)}$-etiocholenic acid, 100 cc. of dry ether, 2.0 cc. of thionyl chloride and 2 drops of dry pyridine stood at room temperature for 3 hours. The solvent was distilled in vacuum at 30°, and to the residue was added 5 cc. of benzene, which was also distilled *in vacuo*.

A methylmagnesium bromide solution from 1.04 g. of the metal in 150 cc. of ether was stirred at the reflux temperature with 4 g. of dry cadmium chloride until the Gilman test became negative (35 minutes). The ether was distilled, 60 cc. of benzene was added, and 25 cc. of this solvent was distilled and replaced by fresh benzene. The acid chloride, dissolved in 50 cc. of benzene, was added rapidly, and the mixture was stirred and boiled for 1.5 hours. After an additional 3-hour period at room temperature the mixture was decomposed with ice-water and acid.

The product was inhomogeneous and was eventually chromatographed on alumina. The most readily eluted fraction (855 mg., 27%) was **3α-acetoxy-$\Delta^{9(11)}$-pregnene-20-one**, m.p. 81–84°. The compound crystallizes as leaflets from dilute methanol, or from ether–petroleum ether; m.p. 84.2–85.1°, $[\alpha]^{21}D$ +123.2 (3.01% Chf), λ_{max}^{chf} broad band 5.82–5.88 μ, strong broad band 8.0 μ.

Anal. Calcd. for $C_{23}H_{34}O_3$ (358.50): C, 77.05; H, 9.56. Found: C, 77.14; H, 9.79.

A sample of the ketoacetate was deacetylated by boiling it for 1 hour with 0.3 *N* sodium methoxide solution. The product, **3α-hydroxy-$\Delta^{9(11)}$-pregnene-20-one**, crystallizes from dilute methanol or from benzene–ligroin; m.p. 165.9–167.6°, $[\alpha]^{22}D$ +114.2° (1.15% Chf).

Anal. Calcd. for $C_{21}H_{32}O_2$ (316.47): C, 79.69; H, 10.19. Found: C, 79.73; H, 10.14.

The next fraction to be eluted amounted, after recrystallization from ligroin, to 578 mg. (18%) of large prisms, m.p. 111.6–113.6°. After recrystallization from dilute methanol and from ligroin the sample melted at 112.6–113.6°, $[\alpha]^{25}D$ +124.5° (1.81% Chf). The infrared spectrum of this substance was very similar to that of the 3α-acetoxy-$\Delta^{9(11)}$-pregnene-20-one described above; a mixed melting point determination gave the value 110–113.2°. A sample of the 85°-substance (8 mg.) was dissolved in warm ligroin (60–90°), and the prisms deposited on cooling (6 mg.) melted 112.2–113.2°. Attempted conversion of the high-melting modification failed.

Anal. Calcd. for $C_{23}H_{34}O_3$ (358.50): C, 77.05; H, 9.56. Found: (85° Cpd.): C, 77.14; H, 9.79. (113° Cpd.): C, 77.07; H, 9.69.

A sample (112 mg.) of the substance described (m.p. 113°) was hydrolyzed by boiling for one hour with 9 cc. of methanol and 1 cc. of 6 *N* sodium hydroxide. By dilution 96 mg. of a solid, m.p. 146–160°, was obtained. This was recrystallized from methanol–water, and then twice from benzene–ligroin, when prisms of 3α-hydroxy-$\Delta^{9(11)}$-pregnene-20-one resulted, m.p. 165.9–167.6°, not depressed on admixture of a sample of the preparation described above. The infrared spectra of the two samples were completely identical; optical rotation $[\alpha]^{23}D$ +112.5° (1.20% Chf).

The methanol–water filtrate on dilution gave 30 mg. of needles that were recrystallized from benzene–ligroin. The substance melted at 145.2–149.8°, $[\alpha]^{23}D$ +46 (1.12% Chf). The infrared spectrum was very similar to that of the hydroxypregnenone described. The compound was not further studied.

The third fraction to be eluted weighed 792 mg. (24%); crystallization from ligroin and from dil. methanol gave prisms of **3α-acetoxy-20-methyl-$\Delta^{9(11)}$-pregnene-20-ol**, m.p. 150.0–151.6°, $[\alpha]^{23}D$ +44.8° (2.90% Chf); λ_{max}^{chf} 2.90, 5.80 μ.

Anal. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.96; H, 10.23. Found: C, 76.92; H, 10.32.

In another experiment the methylcadmium reaction was carried out without removal of the ether; the methyl ketone, m.p. 78–84°, was obtained in 57% yield and a small amount of the tertiary alcohol, m.p. 149–151°, was also found.

3α-Acetoxy-9α,11α-oxidopregnane-20-one (VI).—To 704 mg. (1.96 millimoles) of 3α-acetoxy-$\Delta^{9(11)}$-pregnene-20-one was added 7.00 cc. of 0.322 *M* perbenzoic acid solution in benzene (1.15 equivalents). The solution was diluted to 10.00 cc. and allowed to stand at room temperature. After 40 hours one equivalent of perbenzoic acid had been consumed. The mixture was worked up and the product was digested with a little ether and collected; 409 mg. (64%, taking into account the aliquots removed for titration), m.p. 177–182°. Recrystallization from absolute ethanol gave needles that withered on standing; m.p. 184.0–185.1° after melting and resolidification at about 177°. The mother liquors were not investigated; $[\alpha]^{22}D$ +93.4° (1.14% Chf).

Anal. Calcd. for $C_{23}H_{34}O_4$ (374.50): C, 73.76; H, 9.15. Found: C, 73.64; H, 9.24.

3α-Hydroxy-9α,11α-oxidopregnane-20-one.—A solution of 125 mg. of the oxido acetate described in 3 cc. of 0.3 *N* sodium methoxide was warmed briefly and allowed to stand at room temperature for 16 hours. Dilution precipitated 96 mg. (86.5%) of the hydroxy compound, m.p. 176.8–178.3°. Recrystallized from dilute methanol the compound melts at 177.4–178.3°, $[\alpha]^{22}D$ +77.4° (2.39% Chf).

Anal. Calcd. for $C_{21}H_{32}O_3$ (332.47): C, 75.86; H, 9.70. Found: C, 75.66; H, 9.64.

3β-Hydroxy-3α,9α-oxido-11,20-pregnanedione (VII).—To a solution of 329 mg. of the hydroxyepoxide described in 3 cc. of acetic acid was added 0.4 g. of chromic anhydride, dissolved in 0.8 cc. of water. The mixture stood at room temperature for 16 hours, excess oxidizing agent was destroyed with bisulfite, and the solvent was removed in vacuum. The residue was taken up in dilute hydrochloric acid and extracted twice with ether. The extract was washed with bicarbonate solution and with water, dried and evaporated, giving 264 mg. of a white solid, m.p. 185–201°. Recrystallization from methanol and from benzene, gave, in two crops, 177 mg. (50%) of the product, m.p. 210–216°. The pure substance, recrystallized from benzene, melts 215.4–217.4° with slight discoloration, $[\alpha]^{23}D$ +173.2° (2.41% Chf); λ_{max}^{chf} 2.8–3.0, 5.85–5.87 μ.

Anal. Calcd. for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73. Found: C, 72.57, 72.47; H, 8.81, 8.75.

3,11,20-Triketopregnane (VIII).—The pregnane hemiketal described (127 mg.) was treated with dry hydrogen

bromide in a sealed tube at 0° for 3 hours, as indicated previously for analogous compounds. The product did not crystallize, but it showed a positive Beilstein test, and its infrared spectrum exhibited a broad band in the carbonyl region (5.78–5.84 μ, two bands barely resolved) and a band at 14.8 μ. Debromination of this material was carried out by heating 103 mg. for 15 minutes on the steam-bath with 1 cc. of acetic acid and a pinch each of zinc dust and sodium acetate. The product was chromatographed on alumina; petroleum ether containing solvent mixtures eluted only oils, but pure benzene eluted 29 mg. (36%) of 3,11,20-triketopregnane, m.p. 150–156°. After two recrystallizations the sample melted 156.8–158.6°, $[\alpha]^{22}D$ +116° (1.11% An); these constants are in reasonable agreement with the literature.[9]

Anal. Calcd. for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15. Found: C, 75.87; H, 9.17.

(9) R. Hegner and T. Reichstein, *Helv. Chim. Acta*, **26**, 721 (1943); J. v. Euw, A. Lardon and T. Reichstein, *ibid.*, **27**, 821 (1944).

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, AND THE DEPARTMENT OF PHYSICS AND BIOPHYSICS, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

A Test of Tritium as a Labeling Device in a Biological Study

BY WALTER G. VERLY, JULIAN R. RACHELE, VINCENT DU VIGNEAUD, MAXWELL L. EIDINOFF AND JOSEPH E. KNOLL

RECEIVED JULY 3, 1952

These experiments demonstrate that an isotopic selection takes place in the over-all direction of the greater retention of the carbon–tritium bond in the utilization of the methyl group of methanol in the biosynthesis of the labile methyl group. A mixture of methanols containing carbon-14, deuterium and tritium has been administered to rats and the isotopic contents of the methyl groups of the choline and creatine isolated from the tissues have been compared with those of the methyl group of the methanol administered. The ratio of deuterium to C^{14} in the choline methyl was 22% of that in the methanol, whereas the ratio of tritium to C^{14} in the choline methyl was 69 to 75% of that in the methanol. Hence one might arrive at quite different interpretations of the possible biological pathways of methanol depending on whether tritium or deuterium was used as the labeling device.

The use of tritium as a label for hydrogen attached to carbon in the study of the reactions of intermediary metabolism has definite attractions since tritium can be detected in high dilution. Both tritium and deuterium are generally considered to be satisfactory tracers for carbon atoms or for the fate of carbon-linked hydrogen atoms when the reactions that such a labeled group undergoes do not involve the cleavage of the carbon–hydrogen bond. When a cleavage of the carbon–hydrogen bond occurs, the possibility always exists that a fractionation of the three hydrogen isotopes may result, since the zero point vibrational energy is smallest for the carbon–tritium bond and largest for the carbon–protium bond (isotope effect). These differences result in a lower activation energy for the rupture of the carbon–protium bond and consequently an increased reactivity for the lightest isotope of hydrogen.

As a step in the direction of finding out whether tritium could be used as a label for hydrogen it occurred to us that a study in the rat of methanols labeled with tritium, deuterium and C^{14} in the methyl group as precursors of the labile methyl group would be of interest. It had already been found that when methanol labeled with deuterium and C^{14} was used as a precursor of the labile methyl group in the rat, approximately one-fourth to one-third of the deuterium appeared in the methyl group of choline, relative to the amount of C^{14} appearing in this group.[1] The utilization of methanol for methyl synthesis was therefore interpreted as occurring through an oxidation and subsequent reduction to the labile methyl group. This was in contrast to the results which had been obtained with doubly labeled methionine in which the deuterium to C^{14} ratio was the same in the choline methyl as in the methyl group of the administered methionine.[2,1]

If in the experiments with methanols containing C^{14}, deuterium and tritium there were no differences between tritium and deuterium, one would expect to find approximately one-fourth to one-third of the tritium in the methyl group of the isolated choline. On the other hand, if more tritium than deuterium should be retained, it would be evidence that selection had occurred. Instead of comparing deuterium and C^{14} in one animal and tritium and C^{14} in another, we decided to test the occurrence of isotopic selection in one animal by the administration of triply labeled methanol, *i.e.*, a mixture of C^{14}-methanol, deuteriomethanol and tritium-labeled methanol. The latter method made it possible to eliminate the uncertainties introduced by the biological variations accompanying the separate employment of deuterium and tritium labels in different animals.

Experimental

Synthesis of Tritium-labeled Methanol.—β-Naphthoic acid was dissolved in peroxide-free, dry dioxane, and tritiated water was added to the solution. Water and dioxane were then removed by distillation *in vacuo*. The carboxyl-tritiated β-naphthoic acid, dissolved in dry ether, was poured into an ethereal solution containing an excess of diazomethane prepared from nitrosomethylurea.[3] The excess of diazomethane and ether was then evaporated. The tritiated methyl β-naphthoate was then transferred to a glass tube; a slight excess of powdered, dry potassium hydroxide was added and mixed with the ester, and some ordinary methanol was added as a carrier. The sealed tube was heated in a boiling water-bath for several hours. The tube was then opened and the methanol was distilled *in vacuo* into a cooled trap.

The methanol so obtained was mixed with deuteriometh-

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